Brazilian Journal of Medical and Biological Research (2005) 38: 895-899
ISSN 0100-879X

Preoperative cobalt^{60} irradiation delays the healing of rectal anastomoses in rats

C.B. Fang, W.A. Klug and P. Capelhuchnik

Departamento de Cirurgia, Faculdade de Ciências Médicas, Santa Casa de Misericórdia de São Paulo, São Paulo, SP, Brasil

Abstract

The healing of colorectal anastomoses after irradiation therapy continues to be a major concern. The authors evaluated the healing of rectal anastomoses in a rat model after a preoperative 500-cGy dose of cobalt^{60} irradiation. Thirty-six male Wistar rats were divided into two equal groups: control (group A), and irradiation group (group B). Group B received a single 500-cGy dose of irradiation, and a rectal resection and end-to-end anastomosis was performed in both groups on the 7th day after irradiation. Parameters of the healing process included bursting pressure and collagen content on the 5th, 7th, and 14th days after surgery. In the irradiation group, the mean bursting pressure on the 5th, 7th, and 14th days was 116, 218, and 273 mmHg, respectively. The collagen content assessed by histomorphometry was 9.0, 20.8, and 32%, respectively. In contrast, the control group had a mean bursting pressure of 175, 225 and 263 mmHg, and a collagen content of 17.8, 28.1, and 32.1%, respectively. The adverse effect of irradiation on healing was detectable only on the 5th postoperative day, as demonstrated by lower bursting pressure ($P < 0.013$) and collagen content ($P < 0.008$). However, there was no failure of anastomotic healing such as leakage or dehiscence due to irradiation. We conclude that a single preoperative 500-cGy dose of irradiation delays the healing of rectal anastomosis in rats.

Correspondence
C.B. Fang
Av. Braz Leme, 2242, Bl-1, Apto. 72
02022-020 São Paulo, SP
Brasil
Fax: +55-11-3226-7800
E-mail: colon.reto@terra.com.br

Publication supported by FAPESP.

Received December 9, 2003
Accepted March 1, 2005

Key words
• Irradiation
• Healing
• Rectum

Introduction

Preoperative radiotherapy and chemotherapy are used as standard treatment in rectal carcinoma. Although several studies have shown no significant rates of morbidity and mortality, there is controversy about the side effects of preoperative irradiation such as impaired healing (1-16). Only a higher incidence of perineal wound infection or healing delay has been found, but no increased risk of anastomosis failure (12). Friedmann et al. (10) have shown that moderate doses of cobalt^{60} (Co^{60}) irradiation, such as 4500 cGy, do not affect anastomosis healing. However, there are still concerns about the healing of rectal anastomoses (17), which had led Minsky et al. (18) to recommend a diverting stoma. Conflicting results were also reported in experimental studies using high and moderate doses of irradiation (19-30). Heupel et al. (19) and Crowley et al. (20) reported a good outcome of colon and small bowel anastomoses, respectively, using a single 500-cGy preoperative dose of irradiation in dogs. Higher doses, between

2000 and 6000 cGy, have been related to anastomosis failure, ranging from 20 to 80% in experimental models (21-25). However, Meese et al. (26) demonstrated that late effects of irradiation do not preclude the safe construction of low anterior anastomoses using 4000 and 5000 cGy preoperative irradiation doses in dogs. Degges et al. (23) reported low bursting pressure using a 4500-cGy irradiation dose in rats. If an optimal timing between irradiation and surgery was followed no difference was found. Weiber et al. (27) evaluated the healing of lower left anastomoses in rats using a 2000-cGy preoperative dose of irradiation and obtained similar results such as anastomotic complications, breaking strength and hydroxyproline content of the suture.

There still are concerns about preoperative radiotherapy even when low doses are used and there are no experimental studies in the literature showing the effects of low dose irradiation on the healing of rectal anastomoses. We therefore performed an experimental study using a single dose (500 cGy) of Co^{60} irradiation to evaluate its effects on rectal anastomoses healing.

Material and Methods

Thirty-six male Wistar rats were randomly and equally assigned to a control group and a irradiation group. A Theratron Jr irradiation device (Theratronics International, Ltd., Knanta, Ontario, Canada), Co^{60} 1.2 mev - 9.6 x 10^{12} (260 CI), was used to apply a single 500-cGy dose to the pelvis at 3 cm of depth for 30 min.

A laparotomy was performed on the 7th day after Co^{60} irradiation under ether anesthesia. In a standardized procedure, rectal dissection was performed to the upper border of the seminal vesicle and a 1-cm rectal segment was excised. Then, an end-to-end single layer anastomosis was performed with full thickness interrupted 6-0 mononylon sutures. The rectal specimens were fixed and stained with hematoxylin and eosin for histological study of the effect of the 500-cGy dose of irradiation.

Healing parameters were assessed in 6 rats from each group on the 5th, 7th, and 14th days. The animals were anesthetized with ether and the abdominal cavity and rectal anastomosis were examined. The rectal segment 1.5 cm below the anastomosis was ligated with 3-0 cotton suture. An incision was performed 3 cm above the anastomosis and a catheter was inserted into the rectal lumen. Air was insufflated into the catheter and bursting pressure was determined with a manometer. After bursting pressure evaluation, the rectal segment including the anastomotic line was excised for the assessment of collagen content. Specimens were fixed in 10% buffered formalin solution and embedded in paraffin for histologic examination. Three sections were selected, each obtained every 50 sections, and stained with Masson's trichrome to assess collagen content. A histomorphometric technique similar to those previously described by others was employed (31,32). The collagen content of the granulation tissue along the anastomotic line was measured by counting the amount of fiber in 100-dot fields on a microphotograph slide projected at 120X magnification.

Results

The single Co^{60} irradiation dose (500 cGy) was well tolerated by all animals. Histologic findings such as edema, vascular congestion, microscopic ulcerations, and areas of hemorrhage were detected in all animals on the 7th day after irradiation.

There was no healing failure, leakage or abscess in either group.

Bursting pressure

On the 5th day, 3 animals from the control group presented rectal rupture in an area outside the suture line during the assessment of bursting pressure. No animals in the irradiation group presented such an event. After the 5th day, both groups presented rupture in an area outside of the suture line. The results of bursting pressure are shown in Table 1.

Bursting pressure was significantly lower in the irradiation group only on the 5th day ($P = 0.013$), whereas no significant differences between groups were detected on the 7th or 14th day.

Assessment of collagen content

The results of histomorphometric analysis are shown in Table 2. Collagen content was significantly reduced in the irradiation group only on the 5th day, while no significant differences were found on the 7th or 14th day.

Discussion

There are no clear definitions of a low or high dose of irradiation. The dose used in radiotherapy influences the results and side effects. Many radiotherapy schemes are used, and the concept of nominal standard dose ($NSD = D \times N^{0.24} \times T^{0.11}$, where D = dose, N = number of fractions, and T = overall time) is used in order to standardize the effects of irradiation. We chose a single Co^{60} irradiation dose of 500 cGy to avoid the influence of fractionation and time.

The single 500-cGy dose was well tolerated by all animals. Histologic findings such as edema, vascular congestion, microscopic ulcerations, and areas of hemorrhage were found in all animals on the 7th day. Similar abnormalities were also reported by other investigators (20-22,24,25,32-36). The thickening and hyalinization of the arterial walls reported in other experimental studies (19, 22,36) were not observed in the present study, possibly due to the lower dose of irradiation employed by us.

There was no leakage in the present study, but experimental studies using higher doses have shown leakage rates ranging from 20 to 80% (21,22,25,26).

Although bursting pressure is not the most accurate assessment, it is closer to the physiologic condition of leakage by increasing the intra-luminal pressure than the breaking strength assessment. The gain of both breaking strength and bursting pressure is due to the healing process, mainly collagen synthesis. Not only breaking strength but also bursting pressure correlate positively with collagen content. However, bursting pressure is correlated with collagen content only during the first 10 days of the healing process (37-39).

Crowley et al. (20), using a dose of

Table 1. Bursting pressure (mmHg) of irradiated and control groups.

	Control group				Irradiation group		
	5th day	7th day	14th day		5th day	7th day	14th day
	200	240	280		80*	160	240
	190	240	260		80*	220	260
	160*	220	220		120*	220	260
	200	200	300		120*	220	280
	180*	230	280		120*	240	300
	120*	220	240		180*	250	300
Mean	175	225	263	Mean	116	218	273

*Rupture occurred along the suture line. P = 0.013, P = 0.469, and P = 0.294 when the irradiation and control groups were compared on the 5th, 7th, and 14th days, respectively (Mann-Whitney test).

Table 2. Assessment of collagen content by the morphometric index (%).

	Control group				Irradiation group		
	5th day	7th day	14th day		5th day	7th day	14th day
	10.0	25.1	25.1		4.3	12.7	24.4
	14.1	26.4	25.8		8.9	16.6	29.5
	15.3	28.2	28.1		9.0	22.2	33.2
	17.8	28.3	33.2		10.2	22.3	34.6
	21.0	29.9	39.4		10.8	25.1	36.8
	28.3	30.7	40.8		10.9	26.1	38.6
Mean	17.8	28.1	32.1	Mean	9.0	20.8	32.9

P = 0.008, P = 0.322, and P = 0.531 when the irradiation and control groups were compared on the 5th, 7th, and 14th days, respectively (Mann-Whitney test).

1500 cGy in dogs, did not find differences between the control and irradiation groups in animals submitted to small bowel anastomosis and assessed for healing on the 7th postoperative day (21,22). At this postoperative time, many studies have shown higher bursting pressure at the anastomosis site than in surrounding areas (37,38), with rupture consequently occurring in the vicinity of the suture line. This may be explained, at least in part, by Laplace's law: T = P x r, where T is circumferential wall tension, P is transmural pressure, and r is mean vessel radius. The small diameter at the suture line due to thickening of the bowel wall that occurs during the normal healing process explains the lower tension during bursting pressure assessment at this level and higher tension occurs in the vicinity of the anastomosis. Increased collagenase activity has also been observed in the vicinity of the suture line and consequently less collagen content has been found at this site (40). Collagen synthesis begins as early as on the third day. The side effects of irradiation may only delay the normal healing process and assessment would not detect this effect if done after the early phase of healing.

Degges et al. (23) claimed that there is an optimal time interval between irradiation and surgery to ensure maximal colonic healing. They used a 4500-cGy dose of irradiation in rats and found no impaired healing if irradiation was applied 10 days prior to surgery. Biert et al. (29) reported no difference in bursting pressure when irradiation was applied 5 days before surgery. They found stenosis in spite of anastomosis rupture and this overhealing was compensated for by chemotherapy (28). A healing delay only occurred if irradiation was associated with hyperthermia (29).

Impairment of healing caused by a low dose of irradiation may occur only during the early phase, and therefore the evaluation should be done prior to the completion of a seven-day period after surgery. Experimental studies that assess the anastomosis after this time may not detect this effect since we only found low bursting pressure on the 5th day. We conclude that a single 500-cGy dose of Co^{60} irradiation does not affect the outcome of a rectal anastomosis in rats, but may delay the normal healing process.

Acknowledgments

The authors are grateful to Dr. Carlos Marigo, Professor of Pathology, and to Marilda Guedes, Professor of Statistics, Santa Casa School of Medicine of São Paulo, for their contributions in pathology and statistics studies, and to the Support Center for Scientific Publications for editorial assistance.

References

1. Higgins Jr GA, Humphrey EW, Dwight RW, Roswit B, Lee LE & Keehn RJ (1986). Preoperative radiation and surgery of cancer of the rectum. Veterans Administration Surgical Oncology Group Trial II. *Cancer*, 58: 352-359.
2. Stevens Jr KR, Fletcher WS & Allen CV (1978). Anterior resection and primary anastomosis following high dose preoperative irradiation for adenocarcinoma of the recto-sigmoid. *Cancer*, 41: 2065-2071.
3. Roberson SH, Heron HC, Kerman HD & Bloom TS (1985). Is anterior resection of the rectosigmoid safe after preoperative radiation? *Diseases of the Colon and Rectum*, 28: 254-259.
4. Porter NH & Nicholls RJ (1985). Pre-operative radiotherapy in operable rectal cancer: interim report of a trial carried out by the Rectal Cancer Group. *British Journal of Surgery*, 72 (Suppl): S62-S64.
5. Metzger U, Magdeburg W, Largiader A & Gerard A (1985). Preoperative irradiation of rectum carcinoma. Results of a randomized European multicenter study. *Helvetica Chirurgica Acta*, 52: 707-711.
6. Mohiuddin M, Derdel J, Marks G & Kramer S (1985). Results of adjuvant radiation therapy in cancer of the rectum. Thomas Jefferson University Hospital Experience. *Cancer*, 55: 350-353.
7. Cummings BJ (1986). A critical review of adjuvant preoperative radiation therapy for adenocarcinoma of the rectum. *British Journal of Surgery*, 73: 332-338.

8. Fortier GA, Constable WC, Meyer H & Wanebo HJ (1986). Preoperative radiation therapy for rectal cancer. An effective therapy in need of a clinical trial. *Archives of Surgery*, 121: 1380-1385.
9. Duncan W (1987). Preoperative radiotherapy in rectal cancer. *World Journal of Surgery*, 11: 439-445.
10. Friedmann P, Garb JL, McCabe DP, Chabot JR, Park WC, Stark A, Coe NP & Page DW (1987). Intestinal anastomosis after preoperative radiation therapy for carcinoma of the rectum. *Surgery, Gynecology and Obstetrics*, 164: 257-260.
11. Stockholm Rectal Cancer Study Group (1987). Short-term preoperative radiotherapy for adenocarcinoma of the rectum. An interim analysis of a randomized multicenter trial. *American Journal of Clinical Oncology*, 10: 369-375.
12. Gerard A, Berrod JL, Pene F, Loygue J, Laugier A, Bruckner R, Camelot G, Arnaud JP, Metzger U & Buyse M (1988). Preoperative radiotherapy and radical surgery as combined treatment in rectal cancer. *Recent Results in Cancer Research*, 110: 130-133.
13. Gerard A, Buyse M, Nordlinger B, Loygue J, Pene F, Kempf P, Bosset JF, Gignoux M, Arnaud JP & Desaive C (1988). Preoperative radiotherapy as adjuvant treatment in rectal cancer. Final results of a randomized study of the European Organization for Research and Treatment of Cancer (EORTC). *Annals of Surgery*, 208: 606-614.
14. Reed WP, Garb JL, Park WC, Stark AJ, Chabot JR & Friedmann P (1988). Long-term results and complications of preoperative radiation in the treatment of rectal cancer. *Surgery*, 103: 161-167.
15. Kodner IJ, Shemesh E, Fry RD, Walz BJ, Myerson R, Fleshman JW & Schechtman KB (1989). Preoperative irradiation for rectal cancer. Improved local control and long-term survival. *Annals of Surgery*, 209: 194-199.
16. Pahlman L & Glimelius B (1990). Pre or postoperative radiotherapy in rectal and rectosigmoid carcinoma. Report from a randomized multicenter trial. *Annals of Surgery*, 221: 187-195.
17. Mirhashemi R, Averette HE, Estape R, Angioli R, Mahran R, Mendez L, Cantuaria G & Penalver M (2000). Low colorectal anastomosis after radical pelvic surgery: a risk factor analysis. *American Journal of Obstetrics and Gynecology*, 183: 1375-1379.
18. Minsky BD, Cohen AM, Enker WE & Paty P (1995). Sphincter preservation with preoperative radiation therapy and coloanal anastomosis. *International Journal of Radiation Oncology, Biology, Physics*, 31: 553-559.
19. Heupel HW, Veinberg A & Hunphrey EW (1966). The effect of preoperative Roentgen therapy upon the tensile strength of rectosigmoid anastomoses in dogs. *Radiologia Clinica et Biologica*, 35: 129-140.
20. Crowley LG, Anders CJ, Nelsen T & Bagshaw M (1968). Effect of radiation on canine intestinal anastomoses. *Archives of Surgery*, 96: 423-428.
21. Bubrick MP, Rolfsmeyer ES, Schauer RM, Feeney DA, Johnston GR, Strom RL & Hitchcock CR (1982). Effect of high-dose and low-dose preoperative irradiation on low anterior anastomoses in dogs. *Diseases of the Colon and Rectum*, 25: 406-415.
22. Schauer RM, Bubrick MP, Feeney DA, Johnston GR, Rolfsmeyer ES, Strom RL & Hitchcock CR (1982). Effect of low-dose preoperative irradiation on low anterior anastomosis in dogs. *Diseases of the Colon and Rectum*, 25: 401-405.
23. Degges RD, Cannon DJ & Lang NP (1983). The effects of preoperative radiation on healing of rat colonic anastomoses. *Diseases of the Colon and Rectum*, 26: 598-600.
24. Blake DP, Bubrick MP, Kochsiek GG, Feeney DA, Johnston GR, Strom RL & Hitchcock CR (1984). Low anterior anastomotic dehiscence following preoperative irradiation with 6000 rads. *Diseases of the Colon and Rectum*, 27: 176-181.
25. Morgestern L, Sanders G, Wahlstrom E, Yadegar J & Amodeo P (1984). Effect of preoperative irradiation on healing of low colorectal anastomoses. *American Journal of Surgery*, 147: 246-249.
26. Meese DL, Bubrick MP, Paulson GL, Feeney DA, Johnston GR, Strom RL & Hitchcock CR (1986). Safety of low anterior resection in the presence of chronic radiation changes in dogs. *Diseases of the Colon and Rectum*, 29: 22-26.
27. Weiber S, Jiborn H & Zederfeldt B (1994). Preoperative irradiation and colonic healing. *European Journal of Surgery*, 160: 47-51.
28. Biert J, Wobbes T, Hoogenhout J, de Man B & Hendriks T (1996). Combined preoperative irradiation and direct postoperative 5-fluorouracil without negative effects on early anastomotic healing in the rat colon. *Radiotherapy and Oncology*, 41: 257-262.
29. Biert J, Seifert W, de Man B, Wobbes T, Hoogenhout J & Hendriks T (1996). Combined preoperative irradiation and local hyperthermia delays early healing of experimental colonic anastomoses. *Archives of Surgery*, 131: 1037-1042.
30. Dominguez JM, Jakate SM, Speziale NJ, Savin MH, Altringer WE & Saclarides TJ (1996). Intestinal anastomotic healing at varying times after irradiation. *Journal of Surgical Research*, 61: 293-299.
31. Prandi Filho W, Simões MJ, Kulay Jr L & Goldenberg S (1988). Aspectos morfológicos e morfométricos do processo inflamatório provocado por fio de algodão no subcutâneo dos ratos tratados com diclofenaco sódico. *Acta Cirúrgica Brasileira*, 3: 32-37.
32. Fatureto MC, Simões MJ, Teixeira VPA & Goldenberg S (1989). Aspectos morfológicos e morfométricos do processo inflamatório provocado por fio do categute simples no subcutâneo dos ratos tratados com diclofenaco sódico. *Acta Cirúrgica Brasileira*, 4: 5-9.
33. Stenson S (1969). Effects of proton and Roentgen radiation on the rectum of the rat. *Acta Radiologica Therapy, Physics, Biology*, 8: 263-278.
34. Hubmann FH (1981). Effect of X irradiation on the rectum of the rat. *British Journal of Radiology*, 54: 250-254.
35. Hubmann FH (1982). Proctitis cystica profunda and radiation fibrosis in the rectum of the female Wistar rat after X irradiation: a histopathological study. *Journal of Pathology*, 138: 193-204.
36. Centeno Neto AA (1987). Alterações histológicas retais do rato induzidas por irradiação. Master's thesis, Escola Paulista de Medicina, São Paulo, SP, Brazil.
37. Cronin K, Jackson DS & Dunphy JE (1968). Changing bursting strength and collagen content of the healing colon. *Surgery, Gynecology and Obstetrics*, 126: 747-753.
38. Jiborn H, Ahonen J & Zederfeldt B (1978). Healing of experimental colonic anastomoses. II. Breaking strength of the colon after left colon resection and anastomosis. *American Journal of Surgery*, 136: 595-599.
39. Howes EL, Sooy JW & Harvey SC (1929). The healing of wounds as determined by their tensile strength. *Journal of the American Medical Association*, 5: 42-45.
40. Madden JW & Peacock Jr EE (1967). Measurement of the rate of collagen synthesis in suture rat wound. *Surgical Forum*, 18: 56-57.